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# LIMB REGENERATION IN FIDDLER CRABS: SPECIES DIFFERENCES AND EFFECTS OF METHYLMERCURY

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Brachyuran crabs can autotomize limbs at a pre-formed plane in the basi-ischium and subsequently regenerate them. The limbs grow out in a folded position within a layer of cuticle, and at ecdysis they unfold and become functional. The progress of limb regeneration has been divided into stages by Bliss (1956) who devised the use of a regeneration index [R value, (limb bud length/carapace width) × 100], which facilitates comparisons between crabs of different sizes. After autotomy there is a lag period, followed by basal growth, which establishes the basic organization of the limb. This may be followed by a plateau of anecdysis when the limb is approximately half grown. The final growth phase is proecdysial growth, controlled by the molting hormone, ecdysone (Passano and Jyssum, 1963). This is a phase of rapid growth, which may be followed by a terminal plateau immediately before ecdysis. Since the end point of regeneration is ecdysis, the presence of regenerating limbs can affect the timing of ecdysis. Factors which influence ecdysis can effect regeneration as well. Removal of eyestalks, a source of molt inhibiting hormone, is a standard way of inducing precocious molting. Such animals regenerate limbs rapidly, but generally die at ecdysis. Skinner and Graham (1970, 1972) have shown that multiple autotomy, producing many regenerating limb buds, can cause accelerated regeneration in many species of crabs, also leading to precocious molting. In previous studies (Weis, 1976a, Weis and Mantel, 1976), the fiddler crabs *Uca pugilator* and *Uca pugnax* were used to study the effects of various environmental factors on regeneration and molting after multiple autotomy. It was noted that specimens of *U. pugnax* seemed to regenerate and molt more rapidly than specimens of *U. pugilator*. However, the two species had not been studied at the same time under identical conditions. A comparative study of regeneration in several species of fiddler crabs was then undertaken. This paper is a report of a study on the regenerative rates of the temperate species, *U. pugilator* and *U. pugnax* under identical conditions, as well as the regeneration of several tropical species, *U. rapax, U. thayeri* and *U. speciosa,* as compared to *U. pugilator* from the same location.

Regeneration in fiddler crabs can be affected by environmental pollutants, such as insecticides (Weis and Mantel, 1976) and heavy metals (Weis, 1976b). Methylmercury as a pollutant of the marine environment is of great concern. It can be produced from inorganic mercury by bacterial action and is generally far more toxic than inorganic mercury. The effects of methylmercury on regeneration were tested on *U. pugilator, U. rapax,* and *U. thayeri.* Fiddler crabs, with their estuarine intertidal habitat, are subject to heavy metal pollution, especially in industrial areas.

## Materials and Methods

Specimens of *U. pugnax* and *U. pugilator* were collected from Accabonac Harbor, East Hampton, New York. Autotomy of one chela plus six walking legs was induced by pinching each merus with a hemostat. Crabs were maintained in a small amount of sea water (30‰ salinity) at 24° C in groups of 8–10 crabs per 5-liter plastic aquarium. Groups were maintained in the same location and received 14 hours of light to 10 hours darkness. Groups were arranged to have the same mean carapace width (13 mm) and sex ratio (1 : 1). Crabs were fed twice a week with Purina "Fly Chow," and the water was changed following each feeding period. Limb buds were measured twice a week under a dissecting microscope with a calibrated ocular micrometer. In all cases, the first walking leg, as a representative limb bud, was measured, and values were converted to R values. Mean R values for each group were compared by a *t*-test. Times of ecdysis were recorded for all animals. This limb bud generally attained an R value of about 20 prior to ecdysis.

Specimens of *U. pugilator*, *U. rapax*, *U. thayeri*, and *U. speciosa* were collected in the winter from Boca Raton, Florida, and treated similarly. In these experiments, however, animals were kept in small individual plastic containers in a small amount of diluted sea water (9–10‰ salinity) except when otherwise specified. Eight to ten animals of each species were used in each experiment.

In the methylmercury experiments, methylmercuric chloride (I.C.N. Pharmaceuticals, Plainview, New York) was dissolved first in 0.2% $NaHCO_3$ for a stock solution of 0.1 mg/ml and then added to the water. Control crabs in these experiments received $NaHCO_3$ in the water. Crabs were placed in the appropriate solution immediately after multiple autotomy. After ecdysis, the regenerated limbs were examined under a dissecting microscope.

## Results

### *Comparative regeneration rates*

*Temperate species.* When maintained under identical conditions, *U. pugnax* regenerated more rapidly than *U. pugilator* of the same size (13 mm). Limb buds of *U. pugnax* reached a somewhat larger size before ecdysis, and the crabs molted soon than *U. pugilator* (Table I).

Table I

*R values (mean ± standard error) of first walking legs of* U. pugnax *and* U. pugilator *maintained under identical conditions.*

| Carapace width 13 mm | Days | | | | | |
|---|---|---|---|---|---|---|
| | 7 | 10 | 14 | 17 | 21 | 24 |
| *U. pugnax* | 2.5 ± 0.7 | 7.2 ± 0.9 | 14.2 ± 1.2 | 18.2 ± 1.3 | 20.3 ± 1.2<br>30% molt | 90% molt |
| *U. pugilator* | 1.0 ± 0.4 | 4.8 ± 0.6* | 10.9 ± 0.9* | 15.0 ± 1.0* | 17.4 ± 1.1 | 50% molt |

* $P \leq 0.05$.

*Tropical species.* The first experiment with the Florida crabs was a pilot study with *U. rapax, U. pugilator,* and *U. thayeri* of 14–16 mm carapace width. *U. rapax* regenerated more rapidly than *U. pugilator,* which regenerated more rapidly than *U. thayeri.* By 28 days, 90% of the *U. rapax,* 30% of the *U. pugilator,* and none of the *U. thayeri* had molted. The experiment was repeated with specimens of *U. rapax, U. thayeri, U. pugilator* and *U. speciosa* of 11–13 mm carapace width. The same relative rates were seen (though these smaller individuals completed ecdysis sooner than their larger counterparts in the previous experiment, as expected) and *U. speciosa* was found to regenerate at a rate equivalent to *U. rapax.* The data from the 11–13 mm crabs are seen in Figure 1a. Specimens of *U. thayeri* exhibited a relatively long terminal plateau before ecdysis. However, the overall length reached by limb buds in this species was greater than the others, R being greater than 20, rather than 18–19.

Since previous studies have shown that regeneration in *U. pugilator* is only slightly retarded in crabs maintained in groups (Weis, 1976a), experiments were done on the effects of grouping in these species. Separate groups of ten *U. pugilator, U. thayeri* and *U. rapax* (5 males, 5 females in each group) of carapace width 15–18 were placed in 5-liter plastic aquaria in shallow water. The *U. pugilator,* as expected, continued growth and molted with only slight delay. The *U. thayeri,* however, formed a plateau after basal growth (at $R_1$ of about 10). The *U. rapax* exhibited a lengthened proecdysial phase and terminal plateau (at $R_1$ of about 18); only 33% had molted by 38 or 46 days. The data from this experiment are seen in Figure 1b. At 46 days the specimens of *U. thayeri* and remaining *U. rapax* were placed into separate containers, and within two days 43% of the remaining *U. rapax* had molted. By one week the *U. thayeri* had re-commenced growth, and ecdysis began 18 days after separation.

Similar groups of *U. rapax* in their normal substrate did not exhibit this retardation but regenerated at a rate comparable to those kept in individual containers. Of a group of *U. thayeri* in the mud substrate, half formed a plateau after basal growth, and half continued growing. When specimens of *U. thayeri*

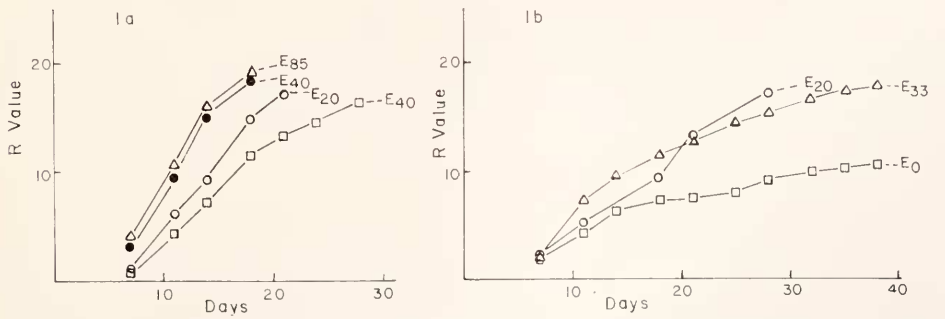


FIGURE 1. Pattern of limb regeneration: a, mean R values of first walking leg of 11–13 mm *U. thayeri* (square), *U. pugilator* (open circle), *U. speciosa* (solid circle), and *U. rapax* (triangle) after multiple autotomy and maintenance of crabs in individual containers; b, mean R values of first walking leg of 15–18 mm *U. thayeri* (square), *U. pugilator* (circle), and *U. rapax* (triangle) after multiple autotomy and maintenance of crabs in groups of ten. "E" with a subscript indicates the time at which that percentage of crabs had undergone ecdysis.

were maintained in groups of four, they again formed a plateau after basal growth, although 25% did reach ecdysis by 46 days. When they were kept in pairs, however, they regenerated and molted at the same rate as animals kept individually.

Smaller individuals of *U. rapax* (carapace width < 16 mm) did not show the long terminal plateau when kept in groups of 10 or in pairs. When specimens of *U. speciosa* (carapace width 10–13 mm) were kept in a group of 10, regeneration continued and ecdysis occurred at the same time as that of crabs kept individually.

*Appearance of regenerated limbs*

It has previously been reported that regenerated limbs of *U. pugilator* are light in color because, although they have the same number of pigment cells as old limbs, their melanophores are more sparsely branched and contain a smaller amount of pigment within them (Weis, 1976a). This very light pigmentation persisted when crabs were kept in the laboratory for up to three months after ecdysis. In some individuals, evidence of banding was visible microscopically. Even when animals were maintained on a dark substrate, the new limbs remained strikingly lighter than old limbs.

Newly regenerated limbs of *U. rapax* and *U. thayeri* were also much lighter in color than old limbs. The new limbs had a banded appearance, with three bands on the merus, two on the carpus and two on the propus. Old limbs were more uniformly dark. In the band regions there were approximately 120 stellate or punctostellate melanophores/mm$^2$, and 40–50 punctate erythrophores/mm$^2$. Chromatophores between the bands were sparse. Within a week the melanophores expanded to a more reticulate condition, making it difficult to distinguish individual cells. The limbs became darker in color and the banding less obvious as melanophores gradually filled in the interband region.

A similar banding pattern was seen in newly regenerated limbs of *U. speciosa*. However, the overall general appearance of the bands was brown in color due to the increased dispersion of the erythrophores, the combination of red and black producing a brown appearance. After several days, the melanophores became more dispersed and appeared in the interband regions, making them narrower, and the limb as a whole, darker, although the banding pattern remained clear. Therefore, in *U. rapax, U. thayeri* and *U. speciosa* the newly regenerated limbs gradually became almost as dark as the original limbs. In *U. pugilator,* however, the light color of the newly regenerated limbs persisted over a considerably longer period of time.

*Methylmercury effects*

*U. rapax.* In the first experiment with *U. rapax*, crabs 10–12 mm in carapace width were exposed to methylmercury at 0.001, 0.01, and 0.1 mg/liter; controls received $NaHCO_3$. Regeneration rates were comparable in controls and all experimental groups, and ecdysis began in all groups by 18 days after multiple autotomy. Subsequently, a group of *U. rapax* (12–16 mm) were exposed to 0.5 mg/liter methylmercury (Table II). Regeneration was inhibited by this concentration, and only 40% of the Hg-exposed crabs reached ecdysis by day 32. The newly regenerated limbs of control crabs had the banding pattern described previously, but

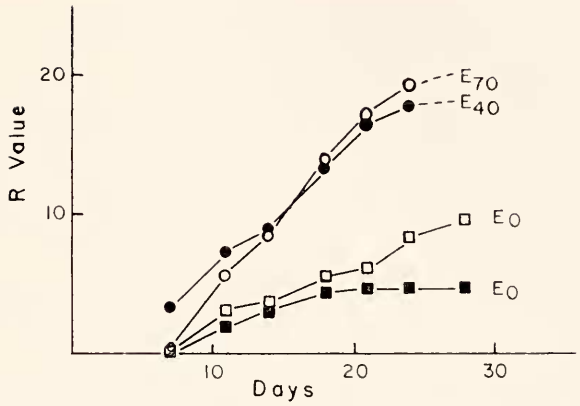


FIGURE 2. Growth of limb regenerates of first walking leg of 12 mm *U. pugilator* in 36‰ salinity (open circle), 9‰ salinity (solid circle), 0.5 mg/liter methylmercury in 36‰ salinity (open square), and 0.5 mg/liter methylmercury in 9‰ salinity (solid square). "E" with a subscript indicates the time at which that percentage of crabs had undergone ecdysis.

the regenerates of the animals that molted in methylmercury were practically devoid of pigmentation. When examined microscopically, erythrophores and leucophores were seen, but the normally more numerous melanophores were absent altogether.

When crabs (14–17 mm) were exposed to 0.3 mg/liter, growth was retarded somewhat, with 25% of the Hg-exposed and 50% of the controls reaching ecdysis by 21 days. Half of the experimental animals had incompletely regenerated one or more limbs, with distal segments lacking. Newly regenerated limbs of all experimental crabs lacked melanin. When this experiment was repeated in full strength sea water (36‰ salinity), 37.5% of the experimental crabs and 60% of the control crabs molted by 21 days. Melanin was again missing in newly regenerated limbs of all Hg-exposed crabs.

*U. thayeri.* In the first experiment with this species, crabs 13–16 mm were exposed to 0.1 and to 0.5 mg/liter methylmercury. The lower concentration had no retarding effect on regeneration. There was, if anything, a slight acceleration of growth. When these animals molted however, the new legs lacked melanin. Erythrophores and leucophores were present, however, and the limbs had a bright red appearance. The animals in 0.5 mg/liter showed practically no limb growth, and at 38 days after multiple autotomy, at which time their R values were only $4.1 \pm 1.8$, they were returned to clean water. Some showed gradual recovery, the first of these crabs reaching ecdysis 26 days later. All of them, however, lacked melanin in the new limbs.

Further experiments were performed to ascertain what length of time the crabs had to be in 0.1 mg/liter to inhibit the production of melanin. Two groups of crabs (15–18 mm) were set up: one was put into 0.1 mg/liter until the animals reached R values of about 10, then transferred to clean water; the other group was put into clean water until they reached R values of about 10, then they were transferred to 0.1 mg/liter. Crabs in this group attained R values of 10 by 12–15 days. At that time pigment was just beginning to appear in some of the

regenerates. In both of these groups only 25% of the animals were lacking melanin in the new limbs after ecdysis.

Additional experiments to investigate this phenomenon involved the effect of 0.1 mg/liter of methylmercury in full strength sea water (36‰). In this experiment 50% of the crabs lacked melanophores in the regenerates. The rest had melanophores, but only one-half to two-thirds as many as in the old limbs. Normally there are about as many melanophores in the new limbs as in old ones.

Another group was placed in clean water for the first week after multiple autotomy, and then, as growth was starting, transferred to 0.1 mg/liter methylmercury. At ecdysis, melanin was absent in limbs of 80% of the animals, and those animals which had melanin had only one-half to two-thirds the normal number of cells.

*U. pugilator.* Experiments were performed to investigate the effects of salinity on the inhibitory action of methylmercury on regeneration. In the first experiment four groups of 12 mm crabs were used. The first two groups were controls in 9‰ and 36‰ salinity, respectively. Group 3 was exposed to 0.5 mg/liter methylmercury in 9‰ salinity, and group 4 was exposed to the same concentration of mercury in 36‰ salinity. As shown in Figure 2, regeneration was greatly inhibited in both full strength and diluted sea water containing methylmercury. Those in full strength sea water grew somewhat more than those in the diluted sea water, however, and the controls in full strength sea water molted somewhat sooner than those in the diluted water. The experimental crabs were analyzed for mercury content by atomic absorption spectrophotometry by Spectrum Laboratories Inc., Fort Lauderdale, Florida. The crabs at lower salinity had taken up more mercury (2 ppm) than those in full strength sea water (0.8 ppm).

In the next experiment four similar groups were organized, except that the concentration of methylmercury was only 0.1 mg/liter. No effect of methylmercury at this concentration, either in full strength sea water or at 9‰ could be observed. After ecdysis, however, 25% of the Hg-exposed crabs in both salinities lacked melanin in the newly regenerated limbs.

Since 0.5 mg/liter at 9‰ salinity had the greatest effect on the initiation of regeneration, a group of crabs (12–14 mm) were kept in clean dilute water until they reached R values of about 10 and then were transferred to 0.5 mg/liter to see if this concentration would inhibit further growth. Controls remained in clean water. The data (Table II) show some retardation of growth after the

TABLE II

*R values (mean ± standard error) of first walking legs of* (A) U. rapax *(12–16 mm) with and without exposure to 0.5 mg/liter methylmercury* (B) U. pugilator *(12–14 mm) transferred from clean water to 0.5 mg/liter methylmercury at $R_1$ of about 10.*

| | | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 7 | 11 | 14 | 18 | 21 | 25 | 28 | 46 |
| A | Control | 2.9 ± 0.8 | 9.7 ± 1.9 | 12.0 ± 1.3 | 17.5 ± 1.7 | 19.7 ± 0.9 | 67% molt | | |
| | 0.5 | 0.8 ± 0.6 | 2.6 ± 1.0 | 4.2 ± 1.2 | 5.3 ± 1.6 | 6.0 ± 1.9 | 7.9 ± 2.6 | | 33% molt |
| B | Experimental | 2.2 ± 0.6 | 5.8 ± 0.6 | 9.1 ± 0.6* | 10.1 ± 0.5 | 13.0 ± 1.0 | 14.7 ± 1.3 | 15.2 ± 1.9 | |
| | Control | 1.1 ± 0.3 | 5.5 ± 0.7 | 8.1 ± 0.9 | 13.2 ± 1.2 | 16.5 ± 1.1 | 20.0 ± 0.5 | 50% molt | |

* Transfer to methylmercury.

addition of the methylmercury. Nevertheless, the crabs did continue growth and did molt under these conditions. Ninety per cent of the crabs in methylmercury had no melanin in their regenerated limbs. In 20%, erythrophores were also lacking. In one individual melanin formed, but melanophores were present in only one-half to two-thirds the normal amount. This was an individual whose R value had exceeded 10 at the time of transfer, and in which melanin had already formed prior to the methylmercury exposure.

In control crabs, melanin normally appears in limb buds at R values of around 10, and the first melanophores to be seen are small groups of cells on the propus and carpus. A few days later erythrophores become visible, and melanophores appear on the merus. The dactyl is the last joint to develop pigmentation.

When newly molted crabs lacking melanin were maintained in clean water, after one to two weeks melanin became visible in some individuals. Melanophores were initially very light brown and gradually became darker and more branched. These limbs became indistinguishable from newly regenerated limbs of crabs that had not been in methylmercury. Melanin did not develop simultaneously in all limbs of a given animal, but melanophores were uniformly distributed throughout the length of a limb, and did not exhibit any proximo-distal gradient.

## Discussion

It has been demonstrated that after multiple autotomy, specimens of *U. pugnax* regenerate and molt faster than *U. pugilator* (Table 1). Fingerman and Fingerman (1974) showed in *U. pugilator* that specimens which molted sooner produced smaller regenerates. Here, however, the *U. pugnax* not only molted sooner, but produced larger regenerates as well. This species also appears "tougher" and shows less mortality in response to handling and experimental manipulation in the laboratory. Engel (1973) has found this species to be more resistant to irradiation than *U. pugilator* or *U. minax*.

*U. rapax* is closely related to *U. pugnax* and was previously considered a subspecies of the same species (Tashian and Vernberg, 1958). It also regenerates more rapidly than *U. pugilator,* as did *U. speciosa.* The latter species is in the same subgenus (*Celuca*) as *U. pugilator*, whereas *U. rapax* and *U. pugnax* are in the subgenus *Minuca* (Crane, 1975). *U. thayeri* (subgenus *Boboruca*) was the slowest to regenerate and generally exhibited a long terminal plateau prior to ecdysis. *U. thayeri* was observed to be much less active than the other species under laboratory conditions and has been reported to have a low general level of social activity in the field (Crane, 1975). Furthermore, it was more difficult to autotomize limbs of this species; more pressure and injury was required. *U. rapax*, on the other hand, autotomized limbs most readily. Frequently, during capture in the field, one or more appendages would be dropped without any injury having occurred. This species also appeared to be the most active. Carapace hardening after ecdysis occurred most quickly in *U. rapax* and last in *U. thayeri.* Thus, there is a correlation between readiness to autotomize limbs and speed of regeneration and molting.

*U. thayeri* had a higher mortality at ecdysis and arrested its regeneration after basal growth when maintained in groups. The arrest of growth in community tanks is adaptive, since crabs molting in such situations are sometimes eaten by

their tank-mates. A growth arrest was seen in the land crab, *Gecarcinus*, by Bliss and Boyer (1964) but did not occur in *U. pugnax* or *U. pugilator;* the only manifestation in these crabs was a slight retardation in males when grouped (Weis, 1976a). In *U. thayeri*, however, the plateau occurred in both males and females, but did not occur when crabs were kept in pairs. Specimens of *U. rapax* in a group showed a prolonged proecdysial phase and terminal plateau, another strategy to delay ecdysis and thereby to enhance survival. A delay did not occur when specimens of *U. rapax* were maintained on a substrate which permitted them to dig burrows, or in small individuals. The difference between *U. pugnax* and *U. pugilator* on one hand, and *U. thayeri* and *U. rapax* on the other, may be adaptations to their being temperate or tropical species. Since regeneration is totally inhibited at 16° C (Passano, 1960; Weis, 1976a), temperate species spend most of the year at temperatures at which they cannot regenerate. It is therefore advantageous for them to replace lost limbs rapidly before temperatures drop to levels which will inhibit growth. Tropical species are not subject to low temperatures and can "afford" to arrest growth in group situations to increase their chances of surviving ecdysis. It is worth noting that *Gecarcinus* is also a tropical species. However, *U. speciosa*, a tropical species, did not delay growth when kept in a group. However, all individuals of this species were small, and *U. rapax* of equivalent size also did not delay ecdysis in a group. Similarly Rao (1965) has found in *Ocypode* that growth and molting in large individuals is inhibited by the presence of other crabs, but that of small individuals is not.

Differences between species of fiddler crabs in resistance to environmental stress have been noted (Vernberg and Vernberg, 1975). Many of these differences are differences between temperate and tropical species. For example, *U. rapax* is more resistant to high temperatures than *U. pugnax* or *U. pugilator*, and the latter two species are more tolerant of low temperatures than the tropical *U. rapax*, *U. mordax*, *U. thayeri*, or *U. leptodactyla* (Vernberg and Tashian, 1959).

The relative rates of regeneration observed in this study are not always correlated with the relative rates of larval development. Vernberg and Vernberg (1975) have found that *U. pugilator* larvae develop to the megalopa stage faster than *U. pugnax* at 25° C and 21‰ or 30‰ salinity. However, at 20° C and 30‰ salinity, *U. pugnax* developed faster than *U. pugilator*. The tropical *U. rapax* was much slower to reach the megalopa stage than the other two species, following the general phenomenon that tropical species function more slowly than temperate zone species at a common temperature (Vernberg, 1962). In this regard, the more rapid regeneration of *U. rapax* and *U. speciosa* in the present study is an exception to this general phenomenon.

A seasonal difference in regeneration rate, especially in *U. thayeri*, was noticeable, since control *U. thayeri* used in methylmercury experiments later in the spring (March–April) regenerated more rapidly than those used earlier and showed a shorter terminal plateau. Ecdysis started by 28 days in 16–18 mm *U. thayeri* used later in the spring. The other species also regenerated somewhat more rapidly as spring proceeded. Seasonal differences in regeneration rate correlated with degree of activity observed in the field. For example, in January and February, specimens of *U. thayeri* were never observed outside their burrows, but were so observed later in the spring.

In some locations, the habitat preferences of the species studied herein are not the same. For example, in Accabonac Harbor, specimens of *U. pugnax* were found in a muddy substrate on a vertical incline, whereas specimens of *U. pugilator* were found in a sandy, more horizontal substrate. In general, *U. rapax* and *U. thayeri* are associated with higher levels in mangrove swamps, and *U. pugilator* with sandy beaches. However, in the Boca Raton collection site, all four species were intermingled in a mixture of fine sand and mangrove peat along drainage ditches extending from the Intracoastal Waterway. No stratification or segregation of species could be discerned. Burrows with chimneys, associated with *U. thayeri,* were often found with other species as occupants. Small numbers of *U. burgersi* and *U. leptodactyla* were also present at this site.

In all three species studied, 0.1 mg/liter of methylmercury had no effect on the rate of regeneration or ecdysis. However, this concentration caused a complete inhibition of melanogenesis in *U. thayeri,* partial inhibition in *U. pugilator,* and no inhibition in *U. rapax.* A mercury concentration of 0.5 mg/liter inhibited regeneration to the greatest extent in *U. thayeri* and to the least extent in *U. rapax.* Thus, the order of resistance to methylmercury is *rapax* > *pugilator* > *thayeri.* This is the same as their relative rates of regeneration, as previously noted.

Two separate effects of methylmercury were seen: inhibition of melanogenesis at lower concentrations, and inhibition of limb growth at higher levels. Inorganic mercury has also been found to inhibit limb regeneration in *U. pugilator* at 1.0 mg/liter, but to have no effect at 0.1 mg/liter. At the higher dosage, mortality rates were high (Weis, 1976b).

Shealy and Sandifer (1975) found that mercury delayed molting and extended the development time of larval grass shrimp. A possible explanation for the retardation of development and growth is that methylmercury is a mitotic inhibitor. It has been found to disrupt the spindle apparatus and cause chromosome breakage (Ramel, 1969). Mercury has also been found to decrease the metabolic rate of fiddler crabs (Vernberg and Vernberg, 1972) and to decrease their activity (Vernberg, DeCoursey and O'Hara, 1974). Larval fiddler crabs are much more sensitive than adults (DeCoursey and Vernberg, 1972); levels of mercury as low as 0.0018 ppb reduced survival of zoeae.

The greater inhibition produced by 0.5 mg/liter in diluted (9‰) sea water as compared with full strength sea water corroborates the findings of Vernberg and Vernberg (1972), who showed that fiddler crabs were more susceptible to mercury at low salinities, and that of Vernberg and O'Hara (1972), who noted that adult specimens of *U. pugilator* take up more mercury in the gills at lower salinities. In that study, total body counts of mercury at different salinities were the same, however. In the present study the total body amount of mercury was greater at lower salinity. This may be a difference between inorganic and organic mercury.

Although extensive work has been done on the physiology of chromatophores in Crustacea (Fingerman, 1965, review), the source of new chromatophores in regenerated limbs remains obscure. The absence of melanin in the mercury-exposed crabs may be due to the absence of melanophores *per se* or to the failure of melanophores to synthesize melanin, due to interference with biochemical pathways. Phenylthiourea can inhibit melanin formation in various organisms including crabs (Green, 1964), and chemicals such as sodium azide and cyanide can inhibit

migration of pigment cells in salamander embryos (Child, 1950). Child discussed the possiblity that pigment formation rather than cell migration was inhibited but suggested that it was cell migration, because the pigment cells that did form were as dark as controls, and he saw only very few cells which were slightly pigmented. In the present study intermediate conditions were seen in some experiments. Generally, in partially inhibited crabs, only one-half to two-thirds the normal number of melanophores were observed in the regenerates. Normal regenerates have about the same number of melanophores/mm$^2$ as old limbs from the same individual, although the cells in the new limbs are less branched and have less pigment in them. The reduction in cell number might imply an inhibition of cell migration, or mitotic activity of precursors (methylmercury is a mitotic inhibitor) rather than an inhibition of melanin synthesis. However, the fact that red and white pigment usually did form might indicate that melanin synthesis was specificially inhibited, unless, in some way, the migration of melanoblasts was prevented, but that of erythroblasts and leucoblasts was not. It would seem more likely that the different biochemical pathways of pigment formation would be differentially affected. Furthermore, the delayed appearance of melanin in limbs of newly molted crabs maintained in clean water would indicate a recovery of the enzyme system of melanin synthesis rather than delayed cell migration, particularly since the melanophores were evenly distributed throughout the length of the limbs and did not exhibit a proximo-distal gradient. The cells were originally very pale brown in color and gradually darkened, reflecting progressive melanin synthesis.

In fishes, pituitary hormones necessary for physiological color change are also involved in melanogenesis. Chen, Wahn, Turner, Taylor, and Tchen (1974) found that MSH induces division of melanoblasts and differentiation of melanoblasts to melanocytes in goldfish. In fiddler crabs, individuals lacking eyestalks, a major source of chromatophorotropins, do develop normal pigmentation in regenerating limbs. This, however, does not rule out a role for chromatophorotropins in melanogenesis, since the central nervous system is another source of these hormones (Sandeen, 1950).

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## Summary

1. When kept under identical conditions, *Uca pugnax* regenerates limbs and molts more rapidly than *U. pugilator* from the same location.

2. The tropical species *U. rapax* and *U. speciosa* also regenerate faster than *U. pugilator* from the same location. *U. thayeri* is the slowest to replace missing limbs, the slowest to harden the carapace after ecdysis, and also requires the greatest injury before autotomy will take place.

3. When kept in groups, *U. thayeri* stops regeneration after basal growth and will not molt; *U. pugilator* is only slightly retarded when kept in groups. *U. rapax* is also affected by grouping, showing a lengthened proecdysial phase and terminal plateau, thus also delaying molting.

4. Newly regenerated limbs of *U. rapax, U. thayeri,* and *U. speciosa,* aside from being lighter than old limbs, have a conspicuous banding pattern. This pattern becomes less obvious during the week or two after ecdysis as melanophores move into the interband regions and the limb as a whole darkens due to increased dispersion of pigment in the melanophores. In *U. pugilator* the newly regenerated limbs are very pale in color and remain that way for several months under laboratory conditions, even when animals are maintained on a dark background.

5. When treated with 0.5 mg/liter methylmercury, growth was inhibited entirely in *U. thayeri.* Partial inhibition was seen in *U. pugilator,* and the least inhibition in *U. rapax.* A few individuals of *U. rapax* were able to complete regeneration and molt, but there was no melanin in the regenerated limbs.

6. Inhibition of melanogenesis in regenerated limbs was also seen in *U. thayeri* and to a smaller extent in *U. pugilator* at 0.1 mg/liter methylmercury. The lack of black pigment may be due to an inhibition of cell migration but more likely of melanin synthesis. Some of these crabs developed melanin when kept in clean water after ecdysis.

7. Seasonal differences were noted in all species, but especially in *U. thayeri.* In this species, regeneration occurred much more rapidly in March–April than in January.

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